

The Office Action

The Office Action sets forth the following grounds for rejection: (1) claim 1 is rejected under 35 USC § 102(b), as allegedly anticipated by U.S. Patent 5,728,527 (Singer et al.); (2) claims 2-4, 5, 7, and 9 are rejected under 35 USC § 103(a), as allegedly unpatentable over Singer et al. in view of U.S. 5,776,782 (Tsuiji et al.); and (3) claim 10 is rejected under 35 USC § 103(a) as allegedly unpatentable over Singer et al. in view of Tsuiji et al. and Sixou et al. (*Nucleic Acids Research*, 22, pp. 662-668 (1994)). Claims 6 and 8 are objected to as being dependent upon a rejected base claim.

Discussion of Anticipation Rejection

Claim 1 is rejected under 35 USC § 102(b), as allegedly anticipated by Singer et al. Although applicants disagree with the rejection, claim 1 has been cancelled.

Claims 11-18 are novel over Singer et al. For example, claim 11 includes, among others, the features of canceled claim 2. Singer et al. fails to disclose a selective separation by irradiating light to the live cell group containing live cells having the hybrid and by identifying live cells which cause a change in fluorescence of said fluorescent dye based on formation of the hybrid, and separating the identified live cells selectively from the live cell group.

Further, Singer et al. fails to disclose the step of determining the site of the mRNA that has high accessibility for oligonucleotide probe hybridization. Singer et al. also fails to disclose the design of appropriate probes (i.e., a probe labeled with a fluorescent dye, having a base sequence complementary to the base sequence of the thus determined site). In this respect, the disclosure of Singer et al. is non-enabling. Singer et al. vaguely mentions the detection or other steps of “a specific nucleic acid” in a cell or tissue (see, e.g., Abstract); however, the cited reference fails to specifically teach how this can be done by a person of ordinary skill in the art without an undue burden of trial and error experimentation. Singer et al. fails to disclose a method for detecting a specific or particular mRNA. In Singer et al., oligo dT is introduced into the cell, poly A region of mRNAs is hybridized with the oligo dT, then observation is made (see Example 7). Since poly A is a sequence region that two or three thousand kinds of mRNA commonly have, strictly speaking, detection of “particular” mRNA in living cell is not made in Singer et al.

In order to detect the particular mRNA, the selection of the mRNA site for probe-hybridization is required. The detection of the particular mRNA is impossible without searching and determining this site. However, Singer et al. does not teach this feature.

The instant invention provides such detection and separation: it first attempts to locate a specific site that has high accessibility for oligonucleotide probe hybridization. This may be

done using the method disclosed in the specification. The site at which a probe can hybridize is thus determined based on the structural information of the target mRNA and the hybridization experiments in vitro and in live cells. mRNA is, because of its secondary and tertiary structures, not easily accessible by oligonucleotide probes; therefore, without careful site selection hybridization cannot be executed even if probes have been designed based on the base sequence of the target mRNA.

The present invention discloses in the "Description of the Preferred Embodiments" section "the searching and determining" based on the experimental data. For example, on page 43 of the specification there is a description of "when the probes are designed, it is also important what site in the mRNA probes hybridize to, as well as the number of bases in a as described above. That is, mRNA itself is a molecule with complex secondary and tertiary structure. Thus, even if the probe to be used has a base sequence complementary to a particular site of the mRNA, an obstruction for the probe to hybridize to the site often occurs in the secondary and tertiary structure when the site interacts with other sites of the mRNA. In the present invention, therefore, the sites where the probes hybridize to have be selected."

In addition, the present specification discloses the experimental search for the hybridization site of target mRNA (IL-2 and IL-4 mRNA) in the "Example" section which includes "(3A) Changes in fluorescence spectra by hybridization of fluorescent labeled probes to human IL-2 RNA, (3B) Changes in fluorescence spectra by hybridization of fluorescent labeled probes to human IL-4 mRNA, (4A) Measurement of hybridization efficiency of probes to human IL-2 RNA by HPLC, (4B) Measurement of hybridization on efficiency of probes to human IL-4 RNA by HPLC, (8) Intracellular hybridization between each probe and IL-2 mRNA in human T-cell leukemia strain Jurkat E6-1 cells induced the expression of IL-2 gene, (9) Intracellular hybridization (ISH) of donor probes and acceptor probes with IL-2 mRNA in IL-2 expression-induced cells, (10) Intracellular hybridization when donor probes and acceptor probes were introduced into live IL-2 expression-induced cells." In addition, Fig. 22 and 24 directly indicate that the probe to hybridize with the target mRNA in cell must have the complimentary sequence to the site that show high affinity against oligonucleotide on the mRNA.

Singer et al. merely discloses as the requirement of the oligonucleotide probe for detecting the target nucleic acids in cells "a labeled single-strand oligonucleotide probe under conditions suitable for uptake of said probe and its specific hybridization to complementary nucleic acids in the cell or tissue, said oligonucleotide having a nucleotide sequence complementary to the target nucleic acids (claim 1)." However, the probe does not hybridize with the target mRNA just by meeting above-described requirements only. Therefore, using such probe, the detection of a specific or particular mRNA mostly results in failure.

Claims 12 and 15-18 are dependent upon claim 11. Claims 13 and 14 correspond to canceled claims 6 and 8, which were only objected to for being dependent upon a rejected base claim.

In view of the foregoing, claims 11-18 are novel over Singer et al.

Discussion of Obviousness Rejections

Claims 2-4, 5, 7 and 9 are rejected under 35 USC § 103(a), as allegedly unpatentable over Singer et al. in view of Tsuji et al. Claim 10 is rejected under 35 USC § 103(a), as allegedly unpatentable over Singer et al. in view of Tsuji et al. and Sixou et al. Although applicants disagree, these claims have been canceled.

Claims 11-18 should not be rejected on this basis. As discussed, Singer et al. fails to disclose the features of the presently claimed invention. Tsuji et al. fails to cure the deficiencies of Singer et al. In addition, Tsuji et al. fails to disclose or suggest to those of ordinary skill in the art the probes as recited in the present claims. The Office Action cites Tsuji et al. to show "a probe composed of two probes capable of FRET." While FRET is generally taught in the reference, no probe is described nor suggested. There are specifically disclosed a fluorescent donor molecule (D) and a fluorescent acceptor molecule (A); neither of them is a probe (by definition). For example, streptoavidin tagged with D and streptoavidin tagged with A are disclosed. See column 13, lines 28-35. Streptoavidin tagged with both D and A is also disclosed. Thus, Tsuji et al lacks in teaching FRET probes (or any conventional probes). The Office Action cannot make a *prima facie* case for obviousness. Tsuji et al., which fails to disclose any probes, cannot properly be compared with the instant invention. Further, Tsuji et al. fails to suggest a method for identifying and separating a cell expressing a particular mRNA (with specificity).

There is no motivation in the cited references to combine them; even if a combination is made (which may be possible only by an impermissible hindsight), the combination does not suggest to those of ordinary skill in the art the presently claimed invention, as one or more of the recited features are absent in the combination.

Moreover, the presently claimed invention provides a superior method. The present invention does not require the washing step of the unhybridized probes. In other words, a washing step is unnecessary. The detection step is carried out to identify live cells which cause a change in fluorescence (e.g., by FRET in claim 12). The presence of any unhybridized probe does not interfere with the detection of hybridized probes: the fluorescence emitted by the unhybridized probes (with no change) can be clearly discriminate from the fluorescence emitted by the hybridized probes. As described on page 39 of the specification, "there are some probes which do not form hybrids in the cell, and it is

In re Appl. of ISHIBASHI et al.  
Application No. 09/775,818

necessary to detect the probes forming hybrids selectively as described above. Then, it is preferable to use, as the present probes, those which cause fluorescence changes based on the formation of hybrids. For these probes described above, two kinds of probes labeled with different fluorescent dyes from each other are used as a pair."

The method of Singer et al., in contrast, includes (or requires) a step of "washing the cell or tissue to allow unhybridized probe to exit from the cell or tissue" (see, for example, claim 1). This means that the invention indispensably requires a washing step for excluding the unhybridized probes from the cell, which needs 1 to 2 hours (typically 100 minutes according to Fig. 3) to complete. See also the descriptions of "excess probes are washed from the cells" (column 1, line 58), "cells are washed for about 1 to 2 hours" (column 5, lines 57) and "excess probe that exited from the cells after about 1 to 2 hours incubation in medium" (column 5, line 66).

In view of the foregoing, claims 11-18 are patentable over the cited references.

Conclusion

The application is considered in good and proper form for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

  
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